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Possible compensatory role among chloroplast proteases under excess-light stress condition

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Abbreviations: Col, Columbia; LHCII, light-harvesting complex of photosystem II; PSII, photosystem II; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; *var2*, *yellow variegated2*

The reaction center protein D1 of photosystem II (PSII), known as a primary target of photodamage, is repaired efficiently by the *PSII repair cycle*, to cope with constant photooxidative damage. Recent studies of *Arabidopsis* show that the endo-type Deg protease and the exo-type FtsH proteases cooperatively degrade D1 in the PSII repair in vivo. It is particularly interesting that we observed upregulation of Clp and SppA proteases when FtsH was limited in the mutant lacking FtsH2. To examine how the complementary functions of chloroplastic proteases are commonly regulated, we undertook a high-light stress on wild-type *Arabidopsis* leaves. The result that wild type leaves also showed increased levels of these proteases upon exposure to excessively strong illumination not only revealed the importance of FtsH and Deg in the PSII repair, but also implied cooperation among chloroplastic proteases under chronic stress conditions.

Light energy constantly damages photosynthetic apparatus, and frequently engenders an inhibitory effect on photosynthesis, known as photoinhibition.[1] Photosynthetic organisms have therefore developed various systems that minimize damage to photosynthetic apparatus and which avoid the accumulation of damaged proteins.[2,3] Because a primary target of the damage is the photosystem II (PSII) reaction center protein D1, quality control of PSII (i.e., rapid and specific replacement of photodamaged D1 with de-novo synthesized D1 in the PSII repair cycle) is an important mechanism.[4,5] Numerous studies of the PSII repair cycle have elucidated rapid D1 turnover. In addition, recent studies have revealed that proteases of two types, FtsH and Deg, participate in this process in chloroplasts.[6,7]

FtsH and Deg have been extensively characterized in *Arabidopsis*. FtsH is a zinc metalloprotease that processively degrades membrane proteins.[8] In chloroplasts, FtsH forms a hetero-hexameric complex.[9-11] The *Arabidopsis* mutants lacking a major isoform FtsH2 or FtsH5, which constitutes two representative isoforms in the heterocomplex, show leaf-variegated phenotypes (termed *yellow variegated2*, *var2* and *var1*, respectively) and accumulate high levels of reactive oxygen species.[12-14] Significant impairment of the rate of D1 degradation in the mutant lacking FtsH provided compelling evidence that FtsH is critically important for D1 degradation in the PSII repair.[15-17] As for Deg, five isoforms are identified in *Arabidopsis* chloroplasts: Deg1, Deg5 and Deg8 are localized on the lumenal side, whereas Deg2 and Deg7 are on the stromal side.[18-21] Deg proteases are regarded as cleaving D1 at surface-exposed loops between transmembrane helices under photoinhibitory light conditions.

Our recent study demonstrated that the fragmented D1 polypeptides, which were detectable only in *var2* background under excessively strong illumination (2,500 μmol photons m^{-2} s^{-1}), resulted from Deg protease activities.[22] These results thus provided in vivo evidence that FtsH and Deg degrade photodamaged D1 cooperatively under photoinhibitory conditions, as shown by in vitro studies. We proposed a model by which, in chloroplasts, fundamental D1 degradation is conducted by FtsH at all light intensities and that Deg proteases assist effective D1 degradation by producing additional recognition termini that are accessible to FtsH under high light.

In addition to cooperation between FtsH and Deg in PSII repair, one noteworthy finding was upregulation of several other chloroplastic proteases in *var2*.[22] Clp is a stromal multi-subunit processive protease that is structurally and functionally related to FtsH. Our recent study demonstrated that Clp levels increased in *var2*. Similarly, a thylakoid membrane-bound protease, SppA, was shown to be upregulated in *var2*. It is particularly interesting that a portion of Clp was recruited from stroma to the thylakoid membrane. Given the functional similarity between Clp and FtsH,[23] it is plausible that Clp can be partially substituted for FtsH, suggesting compensation among chloroplastic proteases.

To examine how the complementary functions of chloroplastic proteases are commonly regulated, here we used wild type

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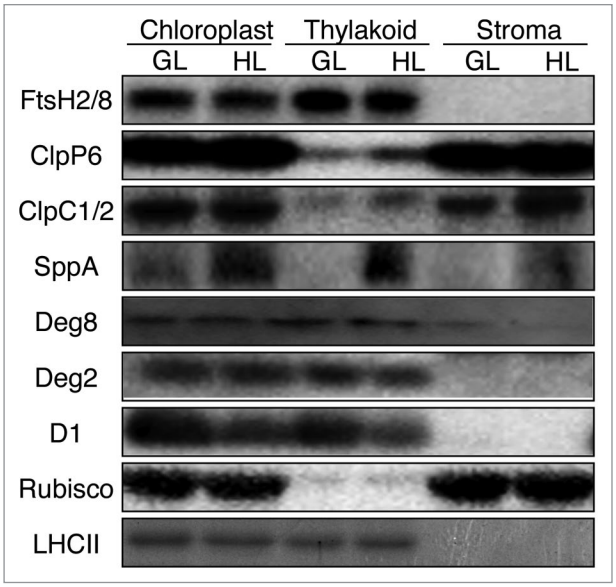

Figure 1. Accumulation and localization of chloroplast proteases under growth light condition and after excessively strong illumination. Chloroplasts were purified using a Percoll step gradient from mature leaves of Col (ca. 6-week-old plants) that were illuminated in growth light (GL) (180 μmol photons m^{-2} s^{-1}) and excessively strong illumination conditions (HL) (2,500 μmol photons m^{-2} s^{-1}) for 6 h. Intact chloroplasts were fractionated into stroma and membrane fractions, according to the protocol as reported previously.[22] A representative immunoblot using specific antibodies (FtsH2/8, ClpP6, ClpC1/2, SppA, Deg2, Deg8, D1 and Rubisco large subunit) and the bands corresponding to CBB-stained LHCII are shown. D1 and Rubisco large subunit were used, respectively, as markers of membranes and stroma. Samples were equally loaded based on chlorophyll content. These experiments were performed three times with similar results.

Columbia (Col) plants instead of *var2*, and observed accumulation of Clp and SppA under excessively strong illumination. Immunoblotting using antibodies against these proteases was performed along with protein fractions from total chloroplasts, stroma and thylakoid membranes. Except for the use of Col and a different light intensity, other experimental condition was identical to those reported in our previous work.[22] First, we compared levels of these proteases after short-time excess light exposure (2,500 μmol photons m^{-2} s^{-1} for 1 h). A significant change in the protease level, however, was never observed. This result made us postulate that constitutive accumulation of damaged proteins in thylakoid membranes, most likely as represented by the situation in *var2*, triggers upregulation of Clp and SppA. To induce constitutive accumulation of damaged proteins in Col, we next examined a chronic high-light stress by prolonged exposure at excess light (2,500 μmol photons m^{-2} s^{-1} for 6 h). As a consequence of this exposure, maximum quantum yield of PSII (Fv/Fm) in Col leaves was decreased to approximately 40% of the initial value before light illumination (Fv/Fm before and after light illumination was 0.83 ± 0.01 and 0.36 ± 0.01, respectively). We found that the prolonged exposure for 6 h caused increased accumulation of Clp and SppA and recruited stromal Clp to thylakoid membranes (**Fig. 1**). These results are consistent with our observation in *var2,* indicating that the compensatory regulation of chloroplast proteases occurs in the wild type.[22] In contrast, we found no significant difference in Deg2 and Deg8 levels in thylakoid membranes after the same light exposure. We observed considerable decrease in D1 protein after the same light exposure, suggesting the excess damage on photosynthetic apparatuses. Whereas, no significant changes of FtsH, Rubisco large subunit and LHC proteins were detected.

It is noteworthy that SppA increased dramatically after treatment, which is consistent with a previous observation that SppA is upregulated by strong illumination.[24] Overall, these results confirm that chronic damage to thylakoid membranes causes changes in the accumulation level of Clp and SppA proteases and the membrane localization of Clp, which implicates the compensation of the chloroplastic proteases in degrading D1 and possibly other proteins in the thylakoid membrane. Although further investigation is necessary, long-term acclimation to light stress and chronic damage to thylakoid membranes might be likely triggers of cooperation among chloroplastic proteases. Consequently, our recent work not only explains cooperative degradation of D1 by FtsH and Deg in the PSII repair; it also elucidates the compensatory control of chloroplast proteases related to high-light acclimation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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